Real-time PCR for detection of fliC gene of E. coli O157:H7 in beef and chicken meat

ABSTRACT

The SYBR Green I real-time PCR assay was used to quantify E. coli O157:H7 in various meat samples. Primers were designed to amplify and quantify the structural flagella (fliC) gene of E. coli O157:H7 in a single reaction. The primer specificity was confirmed with DNA from an ATCC culture of E. coli O157:H7 EDL933 as positive control, autoclaved E. coli O157:H7 EDL933 as negative control (NC) and nuclease free water as non template control (NTC). A direct correlation was determined between the fluorescence threshold (Ct) and the starting quantity of E. coli O157:H7 DNA. A detection limit of 4.71 x 10ï 2 ng/ εl of E. coli O157:H7 DNA equivalent to approximately 1.4 x 10ï 2 CFU of E. coli O157:H7 mlï 1 based on plate counts was determined. Quantification of E. coli O157:H7 in Australian and Malaysian beef, chicken meat, burger and minced beef from the markets was possible when DNA quantity was as low as 1.0 x 10ï 2 ng/εl. These results indicated that the developed PCR assay was suitable for quantitative determination of E. coli O157:H7 in meat samples.

Keyword: Real-time PCR; E. coli O157:H7; Meat; FliC gene